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Technical Note

Roche LightCycler 480
Instrument Setup Instructions
for RT^2 Profiler™ PCR Array, TaqMan® Version

A. Preparation

Before the Experiment (Presetting the Run Template will save time for your run):

1. Make sure the correct thermal block cycler is in place:
   The 96-well block for the F plate format OR the 384-well block for G plate format.
2. Turn on the LightCycler 480 instrument. (The main switch is located at the back of the instrument.)
   Turn on the computer linked to the instrument and log into Window XP.
3. Start LightCycler 480 software.
4. Enter your Username and Password when the Login dialog box appears.
   Click the ☑ button to proceed with the login. The **Overview** window will then appear.
5. Wait for the initiation of the instrument to finish and the two status LEDs on the front of the instrument to become stable. The left LED should become a steady green while the right LED should be a steady orange.
6. In the **Overview** window (see **Figure 1**), click the **New Experiment** button in the **Tasks** area on the left panel to launch the Run module.

Figure 1: Overview Window

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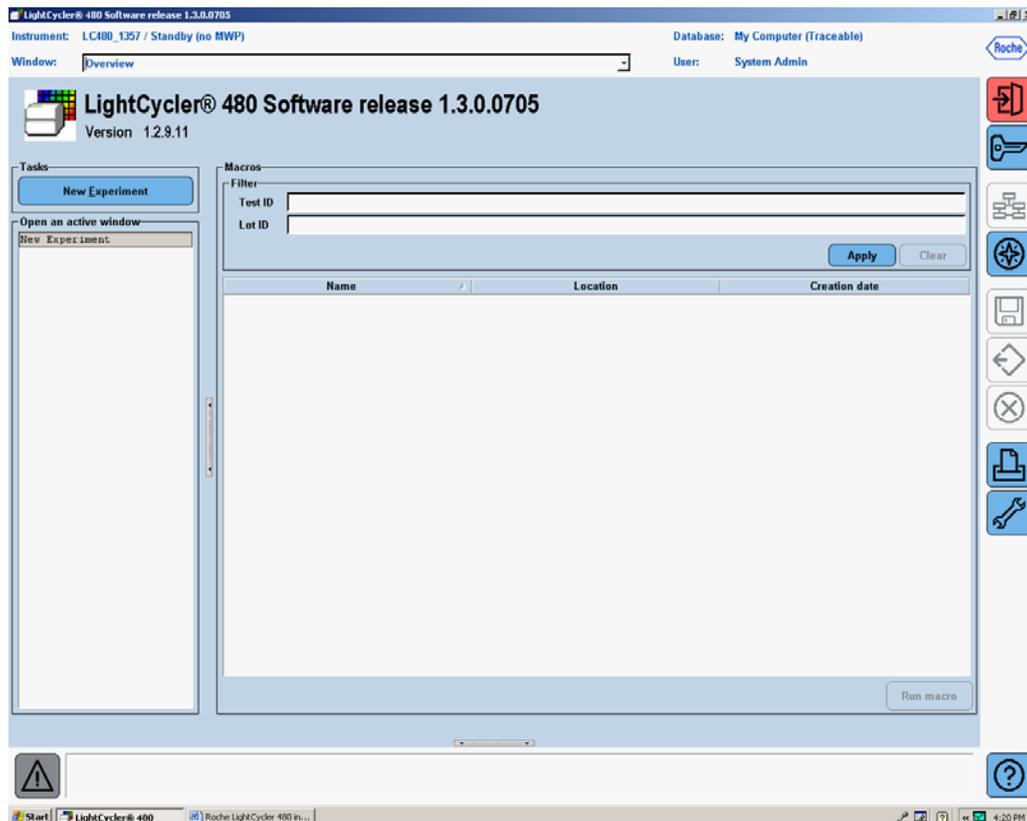
7. In the **Setup** area of the **Run Protocol** tab (see **Figure 2**), specify the following setup parameters:
   Detection Format: **Mono Color Hydrolysis Probe**
   Block Type: Make sure the correct block format is listed (**96** or **384**).
   Plate ID: This feature is optional.
   Reaction Volume: **10** μL for 384-well G plate format
                              25 μL for 96-well F plate format

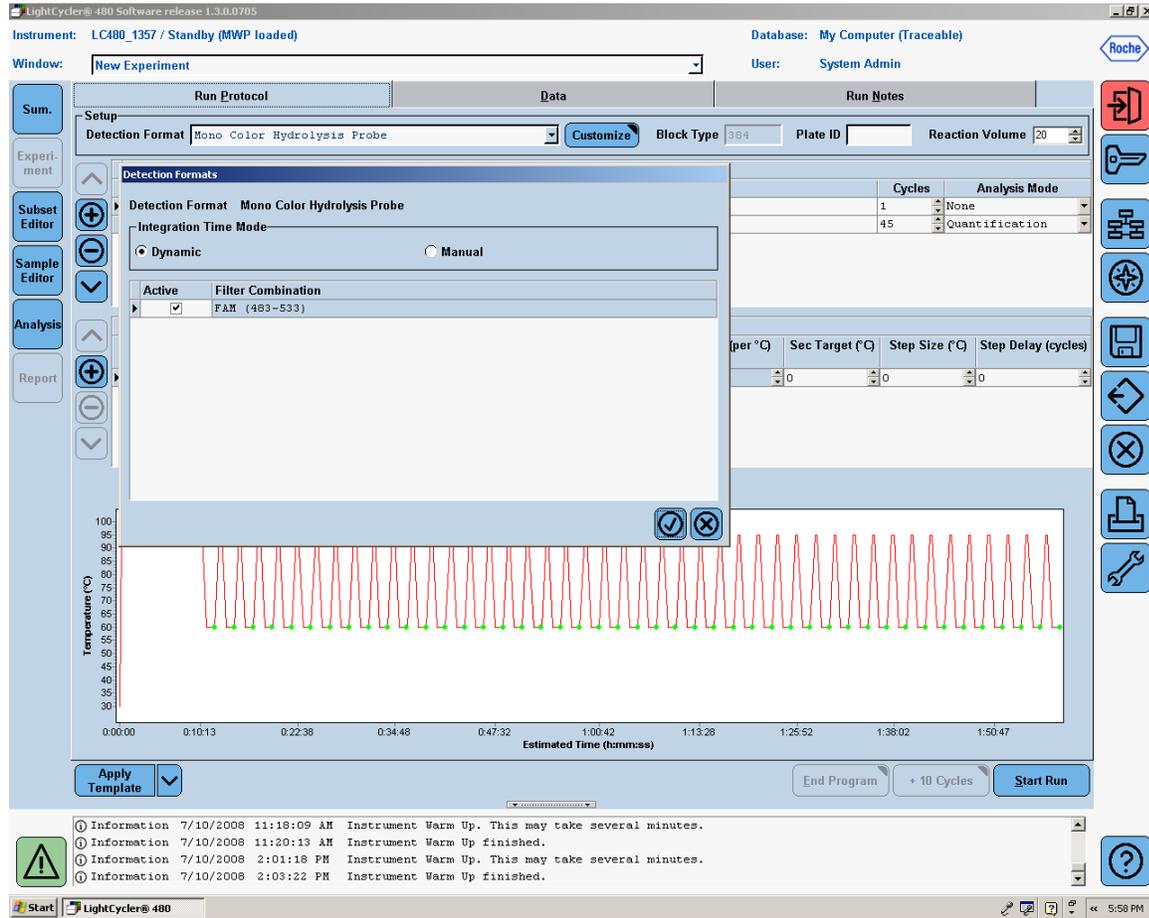**Figure 2: Run Protocol Window**
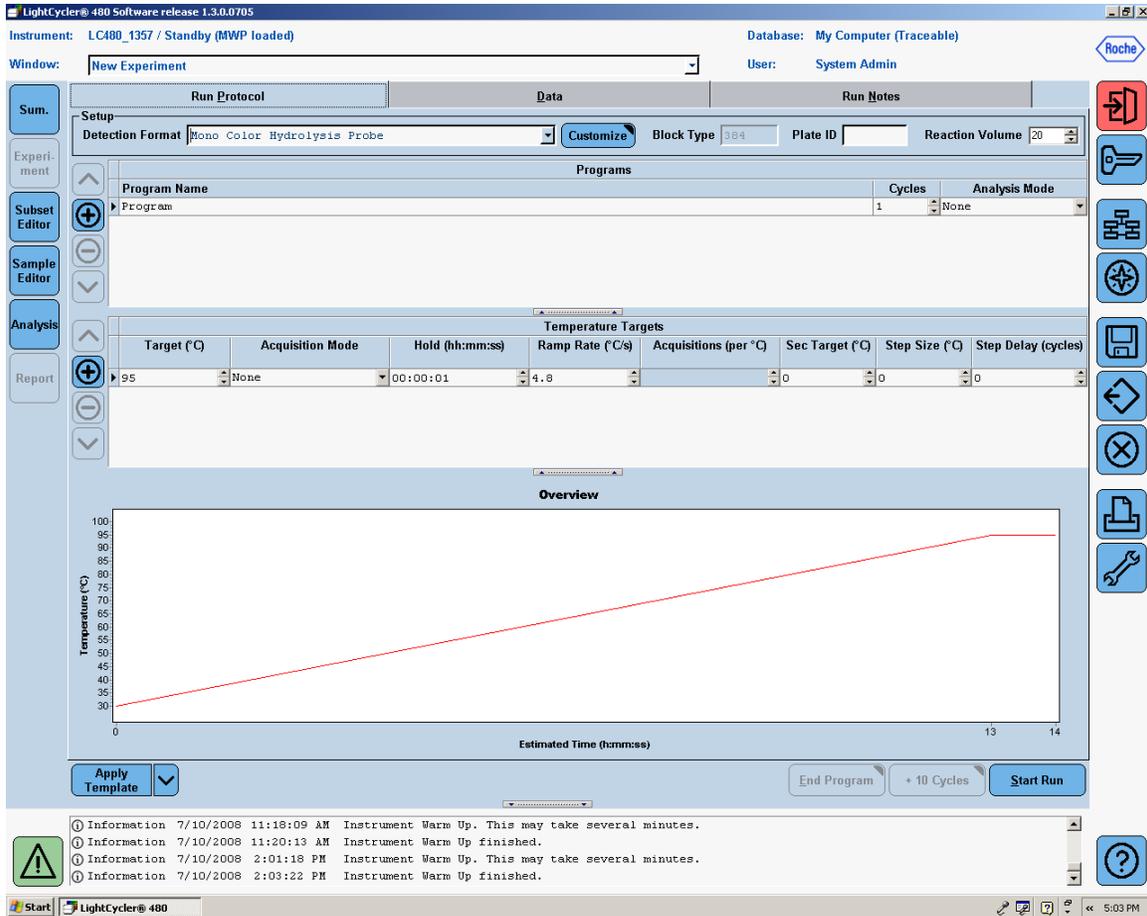
8. Click on the **Customize** button. The **Detection Formats** window should appear.
   Set the Integration Time Mode to **Dynamic** (default) and the Filter combination to **FAM (483-533)**.
   (See **Figure 3**.)

Figure 3: Setting the Detection Format

9. In the **Programs** section, click the ⊕ button twice to add one more programs to the initial program.

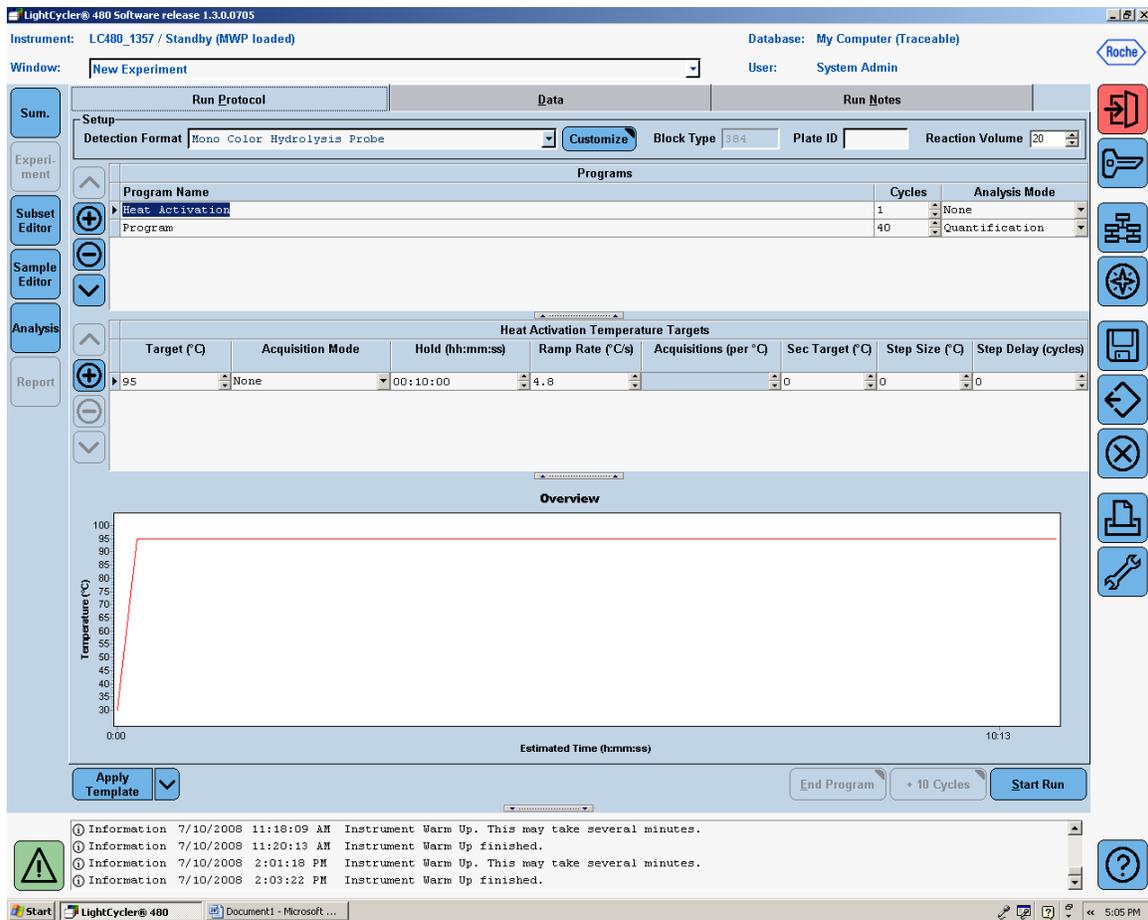10. Change the **Program Name** of the first program to "**Heat activation**".
Input "**1**" for **Cycles** and "**None**" for **Analysis Mode**.
In the **Program Temperature Targets** section, enter the following values for the following parameters:
(See **Figure 4**.)

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisition (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
95	**None**	**00:10:00**	**4.8***		**0**	**0**	**0**

* The default ramp rate for the 384-well block is 4.8 °C/s. The default ramp rate for the 96-well block is 4.4°C/s.

**Figure 4: Setup of the First Program – Heat Activation**

11. Change the **Program Name** of the second program to "**PCR cycle**".
    Input "**45**"** for **Cycles** and "**Quantification**" for **Analysis Mode**.
    In the **Program Temperature Targets** section, click the ⊕ icon to add another segment to the default one. Enter the following values for the following parameters (see **Figure 5**):

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisition (per °C)	Sec Target (°C)	Step Size (°C)	Step Delay (cycles)
95	**None**	**00:00:15**	**1.0**		**0**	**0**	**0**
60	**Single**	**00:01:00**	**1.0**		**0**	**0**	**0**

Figure 5: Setup of the Second Program – PCR Cycle
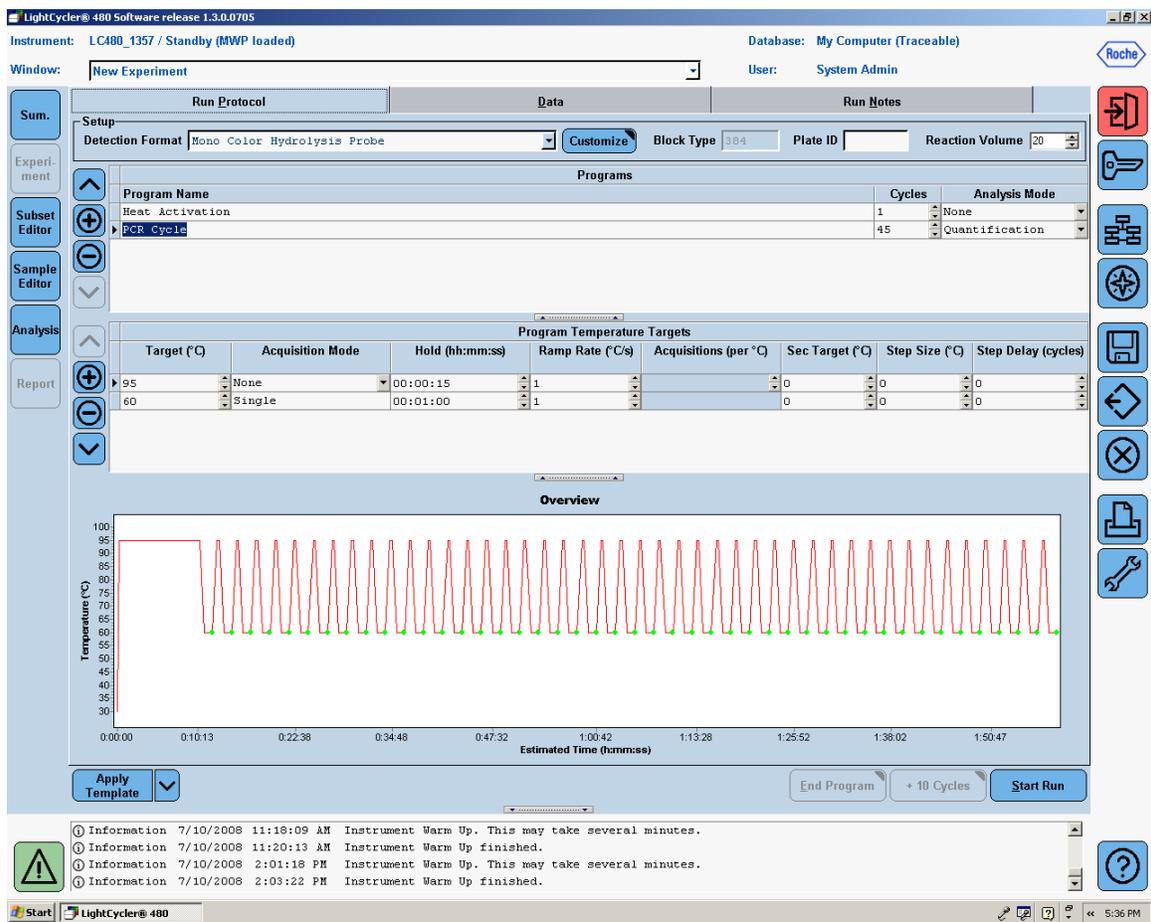

** **NOTE:** PCR is performed for extra five cycles relative to the recommendation in the User Manual (i.e. 45 instead of 40 cycles) to allow the use of the Second Derivative Maximum method available with the LightCycler 480 software. See Data Analysis for further details.

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12. Click the down arrow button (⌄) next to the **Apply Template** button on the lower left corner of the screen. Select the **Save As Template** button (**Figure 6**) to save the run template under the appropriate filename:
"**SABiosciences TaqMan qPCR Array384LC"** for the G plate format
"**SABiosciences TaqMan qPCR Array96LC"** for F plate format.

Figure 6: Saving the Run Template File
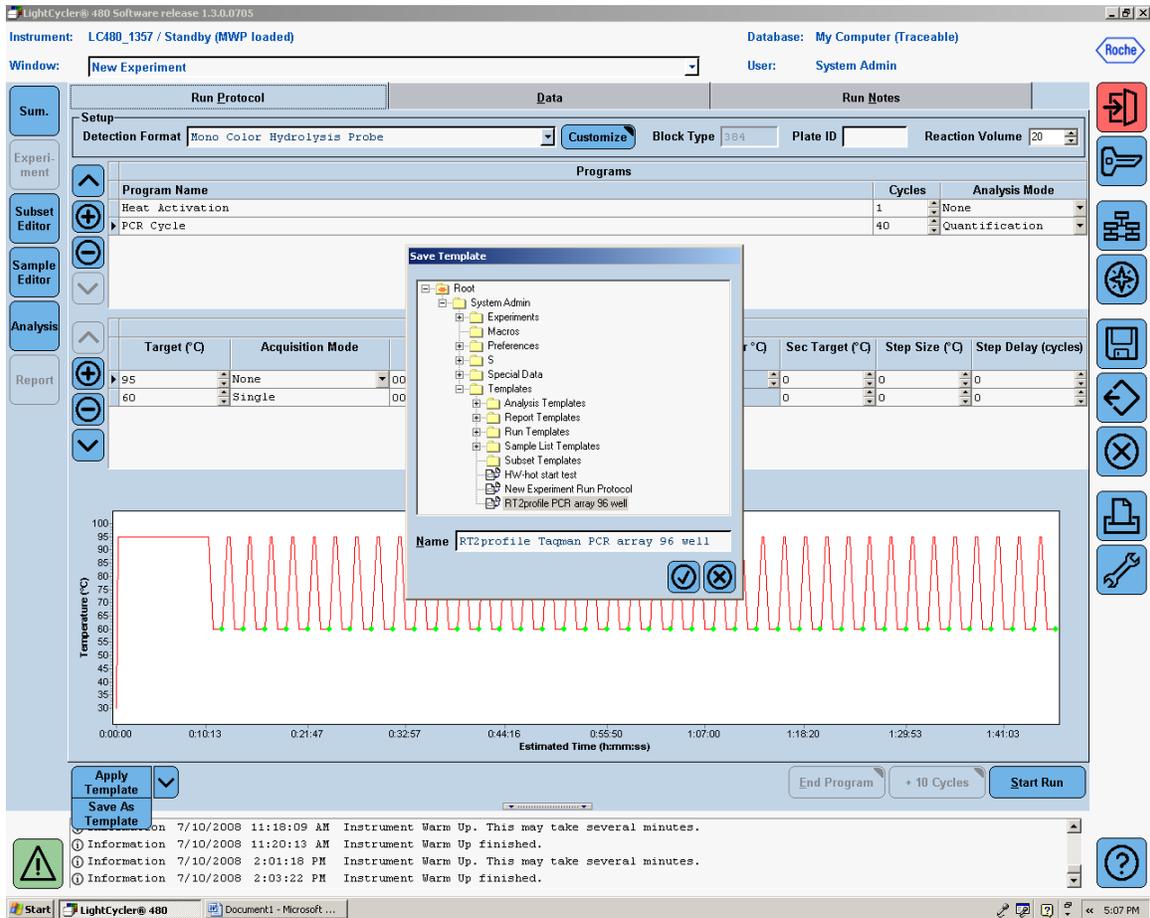
Alternatively, download the appropriate PCR protocol template file, either:
"**TaqManPCRArrayProtocolFile_RocheLC48096Well.ixo**" for the 96-well F plate format or
"**TaqManPCRArrayProtocolFile_RocheLC480384Well.ixo**" for the 384-well F plate format.
Both are available from SABiosciences website at:
http://www.sabiosciences.com/taqmanpcrarrayprotocolfiles.php.

B. Performing Real-Time PCR Detection

To load the PCR Array plate and start the run:

1. When the PCR Array is prepared and centrifuged, bring the plate to the LightCycler 480 instrument to load.

2. Make sure the correct thermal block cycler is in place:
   The 96-well block for the F plate format OR the 384-well block for G plate format.

3. If the instrument is not on, turn it on using the main switch at the back of the instrument.
   Turn on the computer linked to the instrument and log on to Window XP.

4. Start LightCycler 480 software.

5. Enter your Username and Password when the Login dialog box appears.

6. Click the ⊘ button to proceed with the login. The **Overview** window will then appear.

7. Wait for the initiation of the instrument to finish and the two status LEDs on the front of the instrument to become stable. The left LED should become a steady green while the right LED should be a steady orange.

8. Press the push button on the front of the instrument located next to the instrument status LEDs.
   The plate loader will slide out of the right side of the instrument.
   Place the PCR Array plate into the loading frame of the loader with the flat edge pointing towards the instrument and the short edge with beveled corners pointing away from the instrument.

9. Press the plate loading push button again to retract the loader.
   Both of the status LEDs should now turn steady green.

10. In the **Overview** window, click the **New Experiment** button in the **Tasks** area on the left panel to launch the Run module.

11. In the **Run Protocol** tab, click the **Apply Template** button on the lower left corner of the screen.
    Select and double click to open the previously saved **SABiosciences TaqMan qPCR Array384LC"** or **"SABiosciences TaqMan qPCR Array96LC"** run template file based on the block and plate format.

12. Press the **Start Run** button on the lower right corner of the screen to initiate the run.
    When prompted, save the file under a new filename after which the run will begin.

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C. Data Analysis

1. In the **Navigator** window (**Figure 7**), find and open the run file to be analyzed.

Figure 7: Opening the Run File

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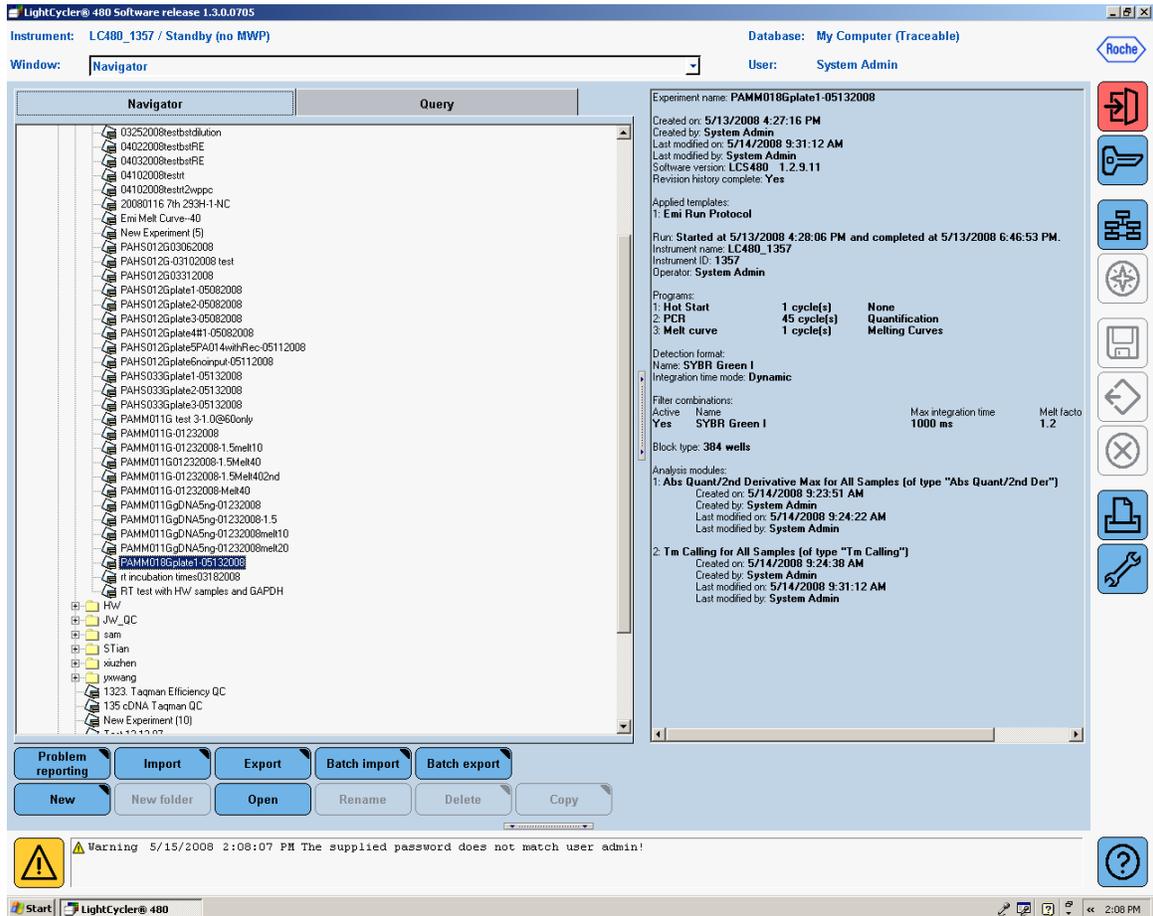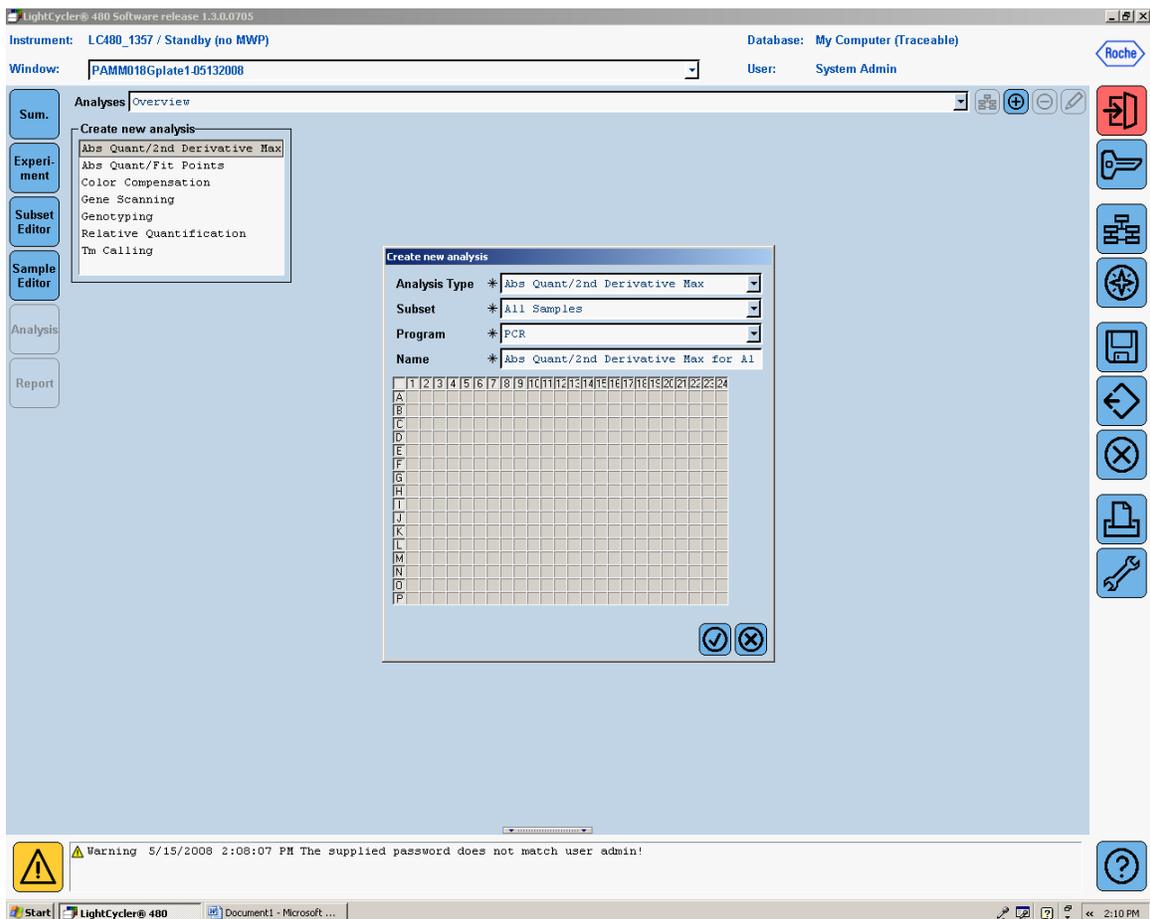
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2. For data analysis using the LightCycler 480 software, we highly recommend using the Second Derivative Maximum analysis method available in this software.

Based on the kinetics of the reaction, this algorithm defines the crossing point (CP) of a reaction as the cycle when its fluorescence reaches the maximum of the second derivative of the amplification curve, in otter words, when the rate of change in fluorescence reaches its maximum. Hence, the crossing point should always be located in the middle of the log-linear portion of the PCR amplification plot. This objective analysis method requires little subjective input from the instrument end-user.

3. Obtain Crossing Point (CP) Cycle Values:
   Once the selected run file is open, click the **Analysis** button on the left module bar.
   The **Analysis** Overview window will open.
   Click "**Abs Quant/2nd Derivative Max**" in the **Create new analysis** list.
   The **Create new analysis** dialog box will then open (**Figure 8**).
   Make sure the **Analysis Type** is "**Abs Quant/2nd Derivative Max**".
   Click ✅ to confirm the selection.

Figure 8: Obtaining the Crossing Point (CP) Cycle Values at the Second Derivative Maximum (SDM)

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4. The Analysis Window with the amplification curves will appear (**Figure 9**).
Click the down arrow button ( ) on the lower right corner of the screen to set the analysis to "**High Confidence.**" Then, click the "**Calculate**" button to generate results.
The calculation may take a few minutes.

Figure 9: Setting Up the Analysis for Calculation of the Crossing Point Cycle Values
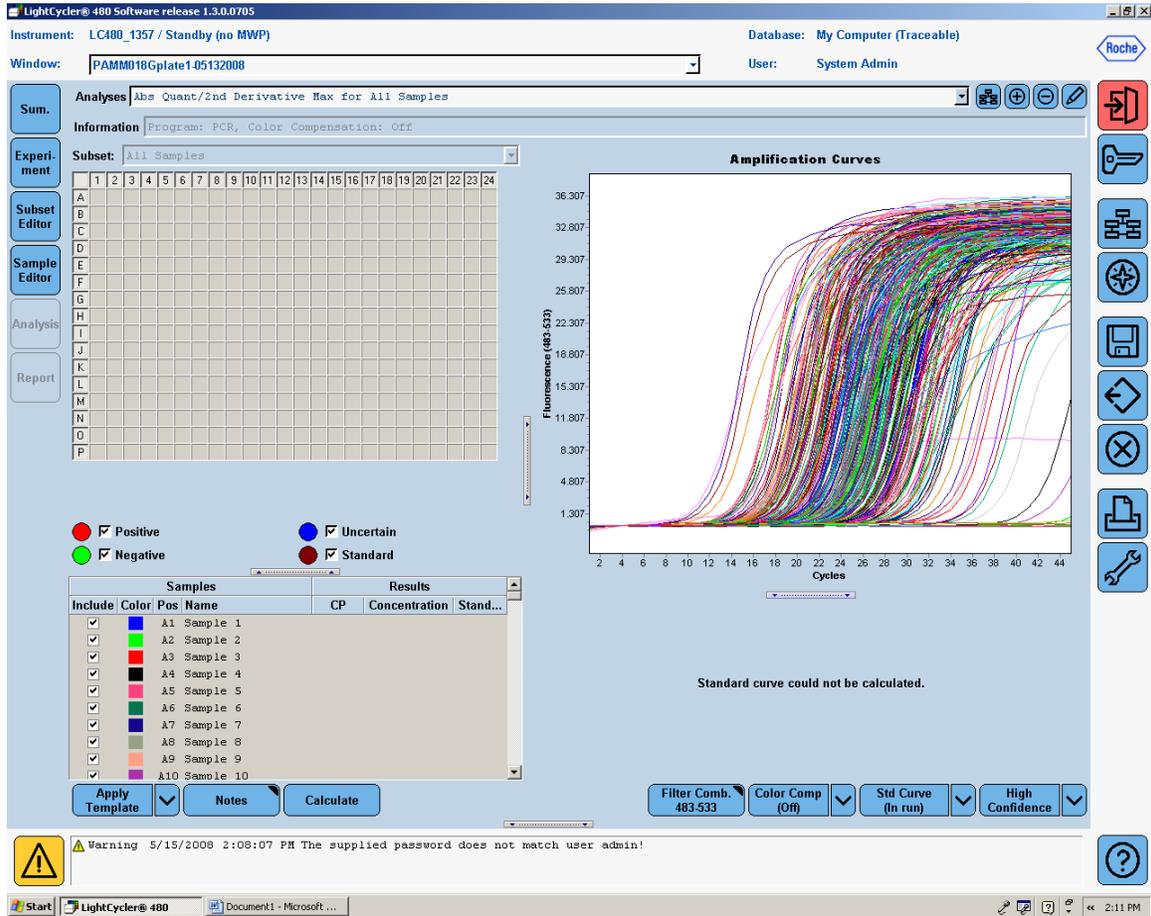
5. Once the CP data is generated, move the mouse over to the result window.
    Right click to highlight the result table.
    Click the "**Export**" button to export the table data (**Figure 10**).
    A "**Save table data**" dialog box will appear. Enter a filename to save the data as a text file.

    Click the 💾 button on the right panel to save the run file now containing the CP data.

6. Open the file that contains the CP values.
    Copy and paste the CP values into SABiosciences Data Analysis Web Portal or download the Data
    Analysis Excel templates from: http://www.sabiosciences.com/pcrarraydataanalysis.php

Figure 10: Exporting the CP Results

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